3-2017

PRELIMINARY SURVEY OF RNA GENOME VIRUSES IN LIMA BEAN

José Evando Aguiar Beserra Jr
Universidade Federal do Piauí

José Wilgney Miguel Teixeira
Universidade Federal do Piauí

Kelvin Josemar Marques Lima
Universidade Federal do Piauí

Marcelo Eiras
Instituto Biológico

PRELIMINARY SURVEY OF RNA GENOME VIRUSES IN LIMA BEAN

José Evando Aguiar Beserra Jr[1], José Wilgney Miguel Teixeira[1], Kelvin Josemar Marques Lima[1] And Marcelo Eiras[2]

[1]Departamento de Fitotecnia, Universidade Federal do Piauí, 64049-550, Teresina, Piauí, Brazil;
[2]Instituto Biológico, Centro de Pesquisa e Desenvolvimento de Sanidade Vegetal, 04014-002, São Paulo, SP, Brazil.

INTRODUCTION: In Brazil, at least nine species of virus have been reported infecting common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* (L.) Walp.). Among these, several stand out because of their predominance in the field: *Cowpea severe mosaic virus* (CPSMV - Secoviridae, *Comovirus*), *Cowpea aphid-borne mosaic virus* (CABMV - Potyviridae, *Potyvirus*) and *Cucumber mosaic virus* (CMV - Bromoviridae, *Cucumovirus*). The viruses that infect cowpea and common bean should be considered potential pathogens for lima bean (*Phaseolus lunatus* L.), mainly because they belong to the same botanical family and are cultivated simultaneously in the same areas. Considering the importance of lima bean and the acknowledged scarcity of information about viruses in this crop, a survey was carried out of RNA genome viruses, which cause mosaic diseases, in lima bean-producing regions in Piauí state, one of the main producers of this leguminous crop in Brazil. The data obtained reveal a predominance of CABMV and CMV, and an absence of CPSMV in the samples analyzed. The collection and analysis of more samples is ongoing, and will supply an overview of the distribution of these pathogens. It will also offer the possibility of detecting other species of virus, because symptomatic samples presented a negative result for the three species evaluated.

MATERIALS AND METHODS: Twelve leaf samples from lima bean plants, exhibiting symptoms of mosaic and leaf deformity (Figure 1) were collected in lima bean fields in the municipalities of Teresina and Várzea Grande, Piauí state, Brazil. Each sample consisted of a plant stem presenting typical symptoms of viral infection. All the samples collected were dried, identified and stored at 20 ºC. The samples were diagnosed in the Instituto Biológico de São Paulo. Twelve samples were analyzed using the plate-trapped antigen - enzyme linked immunosorbent assay (PTA-ELISA) with specific anti-CABMV and anti-CMV polyclonal antiserum, and the RT-PCR protocol (with specific primers for CPSMV) according to Barros et al. (2013). Absorbance at 405 nm was read in an ELISA reader (Microplate Reader 3550- UV, Bio-Rad) in triplicates, after the application of p-nitrophenyl phosphate as substrate. Results obtained were expressed as the ratio of mean absorbance of infected samples to mean absorbance of healthy samples. Samples were considered positive when mean absorbance readings were at least three times as high as negative control absorbance values (Barros et al., 2013). Total RNA was extracted from 0.1 g lima bean leaf tissue in Trizol® medium (Thermo Fisher Scientific) according to the manufacturer's instructions. We conducted RT-PCR using approximately 1 g total RNA and specific primers designed to amplify the protein coat gene of CPSMV (antisense 5'-CTCAAACCCCTGTTGGGACCACA-3'; sense 5'-GGATGAATTTTTGATGGCATGG-3') (Barros et al., 2013). Samples were then placed in a thermocycler and, after initial heating at 94 °C for 5 min, the amplification was conducted as follows: 30 cycles at 94 °C for 1 min, followed by 47 °C for 2 min and 72 °C for 3 min, and a final extension at 72 °C for 7 min. The size of the PCR product expected was 592 bp. Amplified DNA fragments were visualized on agarose gels 1.2% in the presence of ethidium bromide, under ultraviolet light (Sambrook et al., 1989).

RESULTS: Four samples (33.3%) were infected by CABMV, two (16.6%) were infected by CMV (Table 1) and none was infected by CPSMV (data not shown). Mixed infections were not detected. Six samples (50%) with symptoms of mosaic and leaf deformity were not infected by any of the three viruses, which evidences the presence of at least one species naturally present in plants in the field, but which has not yet been described in lima bean. Studies are underway to identify the virus. In the 1980s, in Piauí state, Brazil, Santos et al. (1984) characterized two potyviruses by sorology, morphology and host-range

tests. The authors concluded that the two viruses were distinct from the other bean potyviruses known in the country.

CONCLUSIONS: Based on the diagnosis by ELISA or RT-PCR with anti-serum and specific primers, respectively, the species CABMV and CMV are etiological agents of lima bean mosaic virus in Brazil. Furthermore, there is at least one unidentified species naturally infecting lima bean.

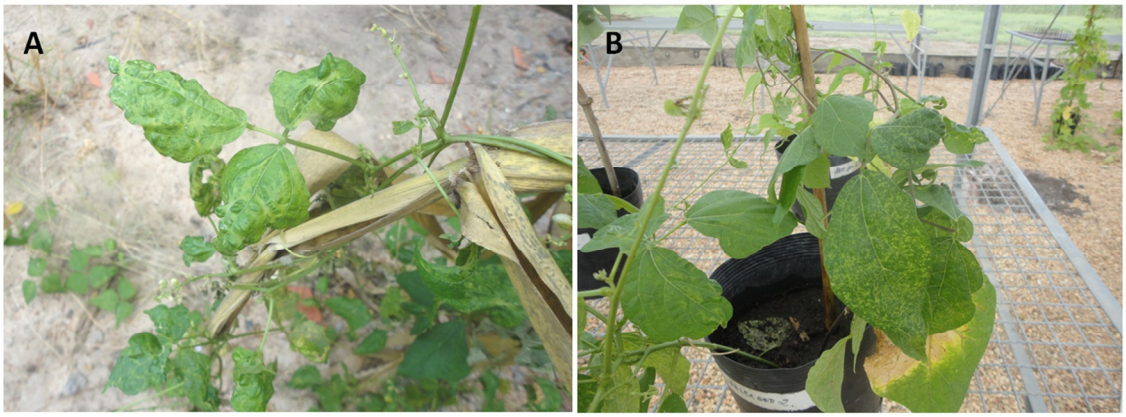

Figure 1. Lima bean (*Phaseolus lunatus* L.) plants infected by CABMV (A) and CMV (B), exhibiting symptoms of mosaic and deformity.

Table 1. Index of lima bean (*Phaseolus lunatus* L.) samples for CABMV (A) and CMV (B) by PTA-ELISA with specific anti-serums. The values within brackets refer to absorbance readings at 405 nm.

A	Sample	Absorbance
	217	+ (A_{405nm} = 1.900)
	228	+ (A_{405nm} = 1.000)
	239	+ (A_{405nm} = 0.900)
	620	- (A_{405nm} = 0.300)
	666	+ (A_{405nm} = 0.900)
	Positive control	+ (A_{405nm} = 0.900)
	Negative control	+ (A_{405nm} = 0.400)
B	Sample	Absorbance
	SC1	- (A_{405nm} = 0.200)
	SC2	- (A_{405nm} = 0.150)
	VG1	- (A_{405nm} = 0.100)
	VG2	- (A_{405nm} = 0.040)
	G28	+ (A_{405nm} = 1.900)
	220	- (A_{405nm} = 0.090)
	250	+ (A_{405nm} = 1.900)
	Positive control	+ (A_{405nm} = 0.600)
	Negative control	+ (A_{405nm} = 0.150)

REFERENCES:

Barros, G.B., Nogueira, M.S.R., Oliveira, C.R.R., Freire Filho, F.R., Ribeiro, V.Q., Veiga, C.F.M., Brioso, P.S.T., Eiras, M. 2013. Obtenção de plantas de feijão-caupi resistentes ao *Cowpea severe mosaic virus* e ao *Cowpea aphid-borne mosaic virus.* Summa Phytopathologica 39: 130-136.

Sambrook, J., Fritsch, E.F., Maniatis, T. 1989. Molecular Cloning, 2 ed. Cold Spring Harbor Press, New York.

Santos, A.A., Lin, M.T., Kitajima, E.W. 1984. Caracterização de dois potyvirus isolados de caupi (*Vigna unguiculata*) no Estado do Piauí. Fitopatologia Brasileira 9: 567-581.